MEMORANDUM

Date: October 22, 2009

From: Alan Trounson, PhD
CIRM President

To: Independent Citizen's Oversight Committee

Subject: Extraordinary Petition for Application DR1-01485

Enclosed is a letter from Dr. Irving Weissman of Stanford University, an applicant for funding under RFA 09-01, CIRM Disease Team Research Awards. This letter was received at CIRM at least five working days prior to the October ICOC meeting, and we are forwarding it pursuant to the ICOC Policy Governing Extraordinary Petitions for ICOC Consideration of Applications for Funding.

As required by that policy, I have reviewed the petition (referencing reviewer comments and the submitted application as necessary) in consultation with the CIRM scientific staff.

The applicant addresses several reviewer criticisms related to the proposed rationale for the antibody therapeutic and asserts an inappropriate bias against the proposed mechanism of action.

We appreciate Dr. Weissman's arguments but upon careful examination of reviewer comments and discussion notes, we believe that reviewers addressed the RFA review criteria appropriately and that their comments were well justified. Although reviewers were very intrigued by the novel and potentially paradigm-shifting mechanism of action, they felt that the preliminary data presented in the application did not fully support a rationale for the proposed therapeutic and they had genuine concerns about whether the therapy would get through to the clinic.

We believe that many of the issues raised by the applicant in this petition represent a scientific difference of opinion or reflect reviewer concerns that were not allayed by the data provided. For example, the applicant contends that a 2-fold difference in expression level of the CD47 antigen (between cancer cells and hematopoietic stem cells) is sufficient for an effective therapeutic window. Multiple, independent expert reviewers asserted this was not an adequate difference based on their own experience. The applicant also contends that observations from clinical experience indicate that macrophage activity in the target patient population will be adequate for the proposed therapy to succeed. However, no information directly addressing macrophage activity in AML patients was provided, and plans for such an assessment were not proposed. The applicant proposes the development of a possible bi-specific antibody but did not provide sufficient information about candidates for the second, targeted antigen (e.g. CD96) to enable reviewers to assess the potential value of this approach.

The need to provide greater specificity by establishing a bi-specific antibody and duel antigen reactivity (CD47 and CD96) makes it challenging for commercialization and clinical application. Nevertheless, the data provided on animal response to the CD47 antibody is important, and efficacy in preclinical trials will be enabling despite the issues of relatively low differences in expression levels and the potential antigen sink that may exist.

Reviewers expressed great confidence in this team of investigators but felt that the proposal was not sufficiently convincing to recommend an award at this time.

This response provides an overall assessment by CIRM staff, based on our careful review of each of the points raised by the applicant. A point-by-point response would require reference to confidential or proprietary information. CIRM staff is prepared to provide that at the ICOC meeting, should a member so request.

The enclosed letter represents the views of its author(s). CIRM assumes no responsibility for its accuracy.

In addition, a copy of the CIRM Review Summary for this application is provided for reference.

STANFORD
SCHOOL OF MEDICINE
Stanford University Medical Center

20 October 2009

To the Chair of the ICOC and the President of CIRM,

We are submitting this extraordinary petition regarding our submission to the CIRM Disease Team RFA as the most significant reviewer criticisms of our proposal had been addressed in the grant and in our linked papers published in *Cell* in July (Jaiswal et al. PMID:19632178; Majeti et al. PMID:19632179). As the reviewer criticisms were covered in these sources, we wonder how this material was reviewed? In contrast to the statements of the reviewers we have clear evidence that CD47 is preferentially targeted on LSC compared to HSC and that a minimum 2-fold expression difference is biologically and clinically significant. We also have clear data that anti-CD47 antibodies can coat all bone marrow cells in the native setting and deliver an anti-leukemia effect without any measurable loss of marrow HSC or progenitors; on this basis, the concern for an "antigen sink" effect is overdone. Furthermore, we have reported extensive toxicity studies of anti-mouse CD47 antibody in normal mice, both in the publications and grant, highlighting the likelihood that anti-human CD47 mAbs will not impart any other major limiting toxicity. Finally, we feel the criticism that anti-CD47 mAbs do not target only LSC in the leukemia, is on the contrary an advantage, as the ability to eliminate both bulk disease and LSC is likely to be clinically beneficial.

Beyond these specific points, we strongly disagree with the criticism that our approach is flawed because "the failure in AML occurs predominantly in the acquired immune system". There is no doubt that enlisting an allogeneic graft vs host (GvH) response improves AML outcomes because some T cell clones are anti-leukemic; avoiding the mortality and considerable morbidity of the GvH response is the art of the bone marrow transplant clinician. However, there is no evidence that the endogenous T cell response was tested and failed, although I personally think it is possible. But it is disturbing and inappropriate for a committee that practices this art to claim the innate immune system is not also able to be very effective if unleashed. We were lucky enough to discover that mouse and human AML express the molecule, CD47, that disarms the innate, and likely the adaptive, immune system by shutting off phagocytosis by both macrophages and dendritic cells. This finding is a paradigm shift. When CD47 is neutralized, macrophages clear most, if not all leukemia cells, and presumably phagocytic immature dendritic cells are permitted to activate T cells with leukemia antigens. The role of the innate immune system in AML had not been revealed prior to our discovery of CD47, accounting for the great interest our findings have received. We can only observe that our grant was about CD47 and blocking antibodies, not about allogeneic transplantation and the belief that T cells from non-tumor bearing allogeneic donors can treat the disease. It is inappropriate that this other topic was even considered.

In the pre-clinical development process, non-human primate toxicity studies must be conducted with the same antibody that will eventually be used in human clinical trials, and this cannot be accomplished without CIRM funding. We have already successfully produced chimeric human-mouse anti-CD47 mAbs that retain binding to human CD47. Scaling production of this and other humanized anti-CD47 mAbs cannot be accomplished without CIRM funding, as the production of FDA-compliant antibody represents a very expensive roadblock. This roadblock will have a multiplicative effect, as this AML trial is also the gatekeeper for trials of synergy with rituximab for human NHL (manuscript submitted) and with herceptin for human bladder and breast cancer. This grant only requests funding for anti-CD47 and synergistic mAbs in AML, but will be the platform for other tumor therapies as well. We understand that you are only considering the AML mono-and dual-mAb and bispecific mAb applications for clinical trials with our UK partners; we are arguing the case for extraordinary reconsideration of funding only on that set of objectives. But we would be remiss not to remind you of our other published, patented, and openly discussed findings.

Over the last year, we have been approached by many companies seeking to license antibody targeting of CD47 for development as a monotherapy in AML. However, it is our

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opinion that as this was a discovery from our cancer stem cell work, it would be best for the people of California for this program to be supported by CIRM funding, where royalties go back to the state and indigent populations can be treated at or near the cost of production. Additionally, our data, and that from others for treatment of lymphoma and solid tumors, indicates that single antibody therapies are generally not curative, but that combination antibody therapies will be more effective. These two points cannot be addressed by licensing the therapeutic development to biotechnology and pharmaceutical companies. We hope the details outlined here will convince CIRM to reconsider and award funding to our proposal.

1. CD47 Expression Difference with Normal HSC Provides Too Narrow a Therapeutic Window:

We have presented multiple lines of evidence to counter this criticism. (1) AML is heterogeneous and we found an association between the FLT3-ITD mutation and increased CD47 (Supplemental Table 1 in Majeti et al.), suggesting that subsets of AML may have higher CD47 expression, which we propose to investigate (Aim 1). (2) In support of the significance of differential expression of CD47 on LSC compared to HSC, we utilized CD47 to prospectively separate HSC from LSC from the same patient sample (Figure 2 in Majeti et al.). In this case, CD47 expression is more than 10 fold higher on LSC indicating that antibody targeting of CD47 will be specific for LSC. (3) The clinical significance of a 2-fold level of increased CD47 expression is indicated by our finding that AML patients with this degree of higher expression had worse clinical outcomes (Grant Figure 3 and Supplemental Figure 3 in Majeti et al.). (4) We have directly investigated the specificity of the effect of anti-CD47 antibody against LSC and normal hematopoietic progenitors. We identified a profound ability of anti-CD47 to induce phagocytosis of LSC both in vitro (Grant Figure 4 – blue samples) and in vivo (Grant Figures 5,6), but not normal CD34+ progenitors (Grant Figure 4 – red samples). (5) Additional data supporting our proposition that a 2-fold difference in CD47 expression has biological significance come from studies of CD47 knockout mice, including our own (Figures 1-3 in Jaiswal et al.), demonstrating that unlike CD47+/+ cells, CD47-/- RBCs, platelets, bone marrow, and HSC were unable to engraft in wild type recipients due to phagocytosis. Our studies (Figure 3 in Jaiswal et al.) also showed that CD47+/- cells, which express half the level of CD47 as wild type cells (Figure 3A in Jaiswal et al.), exhibited defective engraftment. These studies clearly demonstrate that a two-fold difference in CD47 expression is biologically significant.

2. Normal CD47 Expression Will Interfere With Antibody Therapy Serving as an Antigen Sink:

The wide normal tissue expression of CD47 led us to propose to develop bispecific antibodies targeting CD47 in combination with another LSC-specific antigen, both to increase specificity and limit toxicity (Aim 3). Such a bispecific antibody would have anti-CD47 as one part, coupled to anti-CD96 for example. The avidity of each component alone would result in weak binding, but the combination of both antibody specificities on an LSC bearing both targets would create high avidity binding only to LSC. This is the backup alternative to giving two bivalent but monospecific antibodies, where endogenous cells expressing one or the other, but not both, could theoretically be an antigen sink. Despite that theoretical objection, we showed that an anti-mouse CD47 antibody penetrates the bone marrow and coats the cells, and is present in sufficient levels to kill most if not all of the mouse AML. We reported this in the paper by Majeti et al. (Page 291 lines 48-51 and Grant Page 8). Fortunately, the treatment of mouse AML with this anti-mouse CD47 antibody resulted in a statistically significant improved survival (Grant Figure 7). These data strongly suggest that the normal expression of CD47 is not an “antigen sink”, and the mAbs induced no overt evidence of immunological stimulation in these immunocompetent mice, leading to a low likelihood of cytokine storm.

3. Normal CD47 Expression Will Lead to Adverse Secondary Effects:

We feel that multiple lines of evidence indicate that this concern should not prevent pre-clinical development of an anti-CD47 antibody: (1) Monoclonal antibodies directed against the widely expressed EGFR (cetuximab and panitumumab) are approved clinical therapeutics for the treatment of human cancer, delivering clinical benefit with acceptable toxicity in targeting a

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widely expressed protein; (2) We administered a blocking anti-mouse CD47 antibody to normal mice and conducted an extensive evaluation with no evidence of limiting toxicity with the results reported both in our papers and in the grant text on page 8. The dose administered was up to 5 times that needed to coat all bone marrow cells, and was administered for 2-7 weeks. Peripheral blood analysis showed that many anti-CD47 antibody-treated mice developed a mild leukopenia due to isolated neutropenia, but no other hematologic abnormality including anemia and no metabolic abnormalities (Supplemental Tables 3,4 in Majeti et al.). The full coating of endogenous normal mouse HSC and progenitors did not lead to their phagocytosis in vivo (Figure 6 C,D in Majeti et al.). All mice appeared normal during the entire treatment course and one mouse from each group was sent for necropsy which was reported as "all tissues and organs: no significant gross findings". From these studies, we feel that the wide normal tissue distribution will not prevent the anti-CD47 antibody from targeting AML cells in the blood and bone marrow, and will not impart any limiting toxicity to normal CD47-expressing tissues.

4. Macrophage Activity Will Be Inadequate in Patients Exposed to Prior Chemotherapy:

We feel this criticism is countered by observations from clinical experience. Patients treated with chemotherapy do not generally experience a defect in macrophage function other than the monocytopenia resulting from transient myelosuppression. Post-bone marrow recovery, they are not at any increased risk for infections normally countered by monocytes/macrophages, such as Listeria. In AML therapy, bone marrow assessed morphologically on day 14 post-induction chemotherapy shows global hypocellularity with the residual cells composed of histiocytes and macrophages. These patients also recover normal numbers of peripheral blood monocytes along with neutrophils, indicating no global impairment in monocyte differentiation. Furthermore, there is evidence from clinical experience with rituximab in the therapy of NHL to suggest that prior chemotherapy will not severely impair the efficacy of anti-CD47 antibodies. Specifically, it is widely established that rituximab acts by recruiting immune effector cells, including macrophages, to eliminate the target cancer cells. In the treatment of NHL, rituximab is given with combination chemotherapy or in some cases after multiple cycles of chemotherapy, yet it still provides an overall survival benefit. On the basis of these points, we feel it is likely that macrophage function will be sufficient in chemotherapy-treated patients for the anti-CD47 antibody to be effective. Ultimately, we feel that a clinical therapy with anti-CD47 antibodies may be developed as a monotherapy, or possibly as a combination with chemotherapy, macrophage-stimulating cytokines, or myeloid progenitor cell infusions, and have proposed to investigate these methods (Aim 2b).

In summary, we feel that the reviewers were a] inappropriately biased against the proposed mechanism of action and b] overlooked our published data that was presented and linked in our grant submission and c] evidence from current clinical experience that addresses the most significant criticisms levied against our proposal. We should add that at the time of review we had published that human bladder cancer stem cells expressed high levels of CD47, and blocking CD47 enabled them to be phagocytosed and eliminated in vitro. At this point, we have established antibody targeting of CD47 as a viable clinical strategy for the treatment of AML, and following that precedent many other human cancers, as we have identified increased expression of CD47 in breast carcinoma, colon carcinoma, glioblastoma, medulloblastoma, ovarian carcinoma, melanoma, bladder carcinoma, and non-Hodgkin's lymphoma. The next steps for the development of a human clinical therapy are antibody humanization and non-human primate toxicity studies. We believe we will accomplish the goal of an IND filing in the 4 year time period as proposed and hope you will reconsider and award funding for our proposal.

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REVIEW REPORT FOR CIRM RFA 09-01: DISEASE TEAM AWARDS I

DR1-01485: Development of Therapeutic Antibodies Targeting Human Acute Myeloid Leukemia Stem Cells

Recommendation: Not recommended for funding
Final Score:
First Year Funds Requested: $5,052,486
Total CIRM Funds Requested: $19,999,996

Public Abstract (provided by applicant)

Acute myeloid leukemia (AML) is a cancer of the blood and bone marrow that is rapidly fatal within months if untreated. Even with aggressive treatment, including chemotherapy and bone marrow transplantation, five-year overall survival rates range between 30-40%. Evidence indicates that not all cells in this cancer are the same, and that there is a rare population of leukemia stem cells (LSC) that are responsible for maintaining the disease. Thus, in order to cure this cancer, all LSC must be eliminated, while at the same time sparing the normal blood forming stem cells in the bone marrow. We propose to develop therapeutic antibodies directed against surface markers present in much larger amounts on LSC than on the surface of normal blood forming stem cells. We recently identified and validated several such protein markers including CD47, which we determined contributes to leukemia development by blocking the ingestion and removal of leukemia cells by immune system cells called macrophages. In this way, CD47 acts as a "don't eat me" signal on LSC. Moreover, we determined that monoclonal antibodies (mAbs) directed against CD47, able to block its interaction with macrophages, mask the "don't eat me" signal resulting in ingestion and elimination of leukemia in mouse pre-clinical models. We propose a combination of clinical studies, basic research, and pre-clinical development to prepare a therapeutic antibody directed against CD47 and/or other LSC-specific proteins for Initial New Drug (IND) filing with the FDA, and then a Phase I clinical trial to be conducted at {REDACTED} and in the Collaborative Funding Partner country. In collaboration with the pioneering Collaborative Funding Partner country AML Working Group, we will track expression of the LSC proteins in patient samples and correlate with clinical outcomes. This will allow us to identify particular LSC proteins that must be targeted to achieve cure, thereby prioritizing candidate therapeutic antibodies for clinical development. Concurrently, we will conduct basic research and pre-clinical development to prepare these candidates. Basic research during years 1 and 2 will focus on the characterization of anti-CD47 mAb efficacy, investigation of mAb targeting of additional LSC molecules, and determination of efficacy in combinations with anti-CD47. Pre-clinical development during years 1 and 2 will focus on blocking anti-CD47 mAbs, including antibody humanization and large animal model pharmacologic and toxicity studies. Similar studies will be conducted with the most promising antibodies resulting from our basic research. During years 3-4, we will proceed with GMP grade production of the best candidate, followed by efficacy testing in mouse models and large animal models. Finally, in year 4, we will prepare an IND filing with the FDA/MHRA and develop a Phase I clinical trial with this antibody for the treatment of AML. Ultimately, therapeutic antibodies specifically targeting AML LSC offer the possibility of less toxicity with the potential for cure.

Statement of Benefit to California (provided by applicant)

Acute myeloid leukemia (AML) is an aggressive malignancy of the bone marrow with nearly 13,000 new diagnoses annually in the US and 2,200 in the Collaborative Funding Partner country. Current standard of care for medically fit patients consists of several cycles of high dose chemotherapy, and often includes allogeneic hematopoietic cell transplantation. Even with these aggressive treatments, which cause significant morbidity and mortality, relapse is common and the five-year overall survival is 30-40%, but <10% in patients with relapsed or refractory disease or in the majority of AML patients who are over age 65. The goal of this research proposal is to prepare therapeutic antibodies directed against AML stem cell-specific antigens for IND filing with the FDA and a Phase I clinical trial. There are several potential benefits of this research for California: (1) most importantly, this research has the potential to revolutionize current clinical practice and provide a targeted therapy for AML that offers the possibility of less toxicity with the potential for cure; (2) this research will directly contribute to the California economy by funding a contract manufacturing organization to generate and produce GMP-grade clinical antibody, by employing several individuals who will be essential for the conduct of these studies, and through the purchase of equipment and reagents from California vendors; (3) additional clinical and economic

benefits for California will derive from the potential application of clinical agents developed here to a number of other human cancers and cancer stem cells; (4) our animal models indicate that a significant fraction of patients with fatal AML can be cured, resulting in savings on their clinical care plus their return as productive contributors to the California economy; (5) if our therapeutic antibodies show clinical benefit in AML, they will be commercialized, and under CIRM policy, profits derived from treating insured patients and lower cost therapies for uninsured patients, would enrich the state and the lives of its citizens; (6) finally, this research has the potential to maintain California as the national and world-wide leader in stem cell technology.

Review Summary

This proposal is focused on the development of a novel treatment for acute myeloid leukemia (AML). This treatment is based on a therapeutic monoclonal antibody that targets a cell surface molecule, CD47, preferentially expressed on leukemia stem cells (LSCs). These cells are thought to drive leukemia growth and to display elevated resistance to conventional chemotherapeutic agents. Antibody binding to CD47 (or to additional cell surface molecules) is expected to facilitate macrophage phagocytosis and removal of LSCs. The applicant team will develop a humanized blocking anti-CD47 monoclonal antibody and test its efficacy in a mouse xenotransplantation model system. They will also identify other potential cell surface targets on AML LSCs and evaluate the therapeutic value of antibodies specific to these molecules. In later stages of the study, GMP-grade production of the most promising antibodies followed by efficacy and safety testing in appropriate in vivo models will precede preparation of IND filing.

The proposed research is focused on a significant and unmet medical need, as AML is often a rapidly fatal disease with five-year survival rates between 30-40%. Reviewers acknowledged the need for the development and implementation of novel and improved therapeutic strategies. They also believed that the rationale of targeting LSCs was appropriate for AML, particularly since evidence for cancer stem cells in this disease is very strong and widely accepted. Additionally, monoclonal antibody therapies are currently demonstrating efficacy in treatment of a broad range of tumors.

The major focus of the application is on developing potential candidate therapeutic antibodies specific for CD47. Reviewers noted that the applicant did not discuss within the scientific rationale that the central implication of a strategy targeting CD47 suggests that a major, if not the major, determinant of leukemic stem cell maintenance is a failure of innate immunity (macrophage phagocytosis). This is contrary to a very large body of work that suggests the failure in AML occurs predominantly in the acquired immune system, thereby providing a rationale for the well-established allogeneic hematopoietic stem cell (bone marrow) transplantation therapy and the graft versus leukemia effect. In addition, reviewers expressed serious concerns about this target. In particular, they felt that the preliminary data (much of which was recently published) raised considerable questions regarding the validation of CD47 as a compelling target for therapy. A key concern shared by the reviewers was that CD47 expression was shown to be only approximately two-fold higher on AML than normal hematopoietic stem cells (HSC), providing only a very small therapeutic window for targeting cancer cells specifically. In addition, there was little apparent difference in expression level between bulk AML cells and LSCs, again suggesting no ability to specifically target the cancer stem cells. Moreover, reviewers noted that CD47 is apparently expressed at significant levels on many normal tissues, and this could lead to adverse, secondary events or, minimally, interfere with antibody therapy by serving as a considerable antigen sink. Reviewers were encouraged by studies demonstrating that anti-human CD47 antibodies administered to mice engrafted with LSCs led to depletion of AML, but they were not confident that this xenotransplantion model would accurately predict therapeutic responses in human patients.

Another significant reviewer concern about the project's feasibility was related to the proposition that treatment with anti-CD47 antibodies will lead to eradication of AML cells via macrophage phagocytosis. This plan is dependent on patients having normal, robust macrophage activity, but reviewers considered this unlikely in patient populations with later stages of disease who likely have been exposed to prior chemotherapy. The applicant did not address this complication.

Reviewers found the research and development plan to be logically organized but were of mixed opinions as to whether an IND was possible within the proposed time frame even for an anti-CD47

monoclonal antibody. Some reviewers felt that plans to define a critical antigenic LSC profile using serial patient samples to correlate with clinical outcomes was a particular strength of the proposal; others considered this component to be largely superfluous and not needed for the development of an IND application particularly given the sample numbers contemplated for analysis. Another concern was that the proposal lacked adequate discussion of potential pitfalls and alternate plans should substantial roadblocks be encountered, and problems noted in the previous development of other therapeutic antibodies were not discussed. Program milestones were viewed as meaningful and the timeline generally feasible. However, the critical decision for the entire project will be which particular anti-CD47 antibody (or other antibody) to produce for clinical development, and the absence of clearly stated criteria for this decision raised additional concerns about the project's feasibility.

Reviewers viewed the PI, a recognized leader who has made outstanding contributions to the fields of hematopoiesis research and stem cell biology, as a predominant strength of the proposal. The PI's expertise, track record, and capacity to lead the project were strongly acknowledged. Reviewers had serious reservations about qualifications of the Co-PI, a recently appointed junior faculty member with no apparent experience directing large research projects. The Partner PI was recognized as an extremely strong clinical researcher with outstanding experience appropriate to the proposed project. The leadership plan was sufficiently well formulated to ensure coordinated activities across the team, to monitor progress, implement strategic decisions and to resolve any potential conflicts.

The environment and available resources for the proposed studies were considered excellent. Reviewers also viewed the collaboration between the extraordinarily productive groups led by the PI and Partner PI, respectively, as one of the greatest strengths of the application. Collectively, the multidisciplinary research team assembled represents an appropriate mix of basic research, clinical and management skills to ensure successful completion of the project. Reviewers considered the equipment costs for PI and Co-PI to be excessive and felt that the extent and redundancy of equipment requests were unjustified.

This application describes a project to develop therapeutic monoclonal antibodies targeted to LSCs to treat AML. Strengths of the proposal include the outstanding qualifications of the PI and Partner PI, the complementary and well-established collaboration, and the medical need of better treatments for AML. Weaknesses include serious concerns about the validity of the proposed therapeutic target and other issues with project feasibility.

PROGRAMMATIC REVIEW

A motion was made to move the proposal into Tier 1. Key strengths and weaknesses of the proposal were reviewed. Proponents considered the focus on cancer stem cells an important therapeutic advance. Opponents were concerned with the project's feasibility, considered the approach to be an extremely high risk one, and asserted that other applications focused on leukemia have been rated highly. The motion failed. A subsequent motion to move the proposal down to tier 3 carried.

The following scientific Grants Working Group members had a conflict of interest with this application:
Balber, Andrew; Mendez, Ivar